Kidney International, Vol. 8 (1975), p. 135–139

Intrarenal blood flow in carotid sinus nerve stimulation and hemorrhage in dogs

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Intrarenal blood flow in carotid sinus nerve stimulation and hemorrhage in dogs. The present study was undertaken to compare the role of the sympathetic nervous system, in hemorrhage, with that of hypotension in producing renal blood flow (RBF) redistribution. Ten mongrel dogs were prepared for the determination of RBF distribution by injecting ^{85}Kr dissolved in saline into the renal artery to obtain renal radioactivity curves. RBF distribution was determined *a*) at control, *b*) during a 15-min period of hypotension produced by electrical stimulation (10 v—60 Hz) of the left carotid sinus nerve (nerve of Hering), *c*) 15 min after hemorrhage to a blood pressure equivalent to that of stimulation and *d*) during hemorrhage plus a 15-min period of stimulation. Hypotension caused by stimulation left component I blood flow unchanged (at approximately 492 ml/min/100 g) but resulted in an increase in component II flow from 93 ± 8 to 155 ± 20 ml/min/100 g. Hemorrhage caused a 60% reduction in component I blood flow rate, leaving component II unchanged. Partial reversal of hemorrhage effects on distribution of RBF was obtained by restimulation of the nerve of Hering. It appears that RBF distribution, as controlled by the carotid sinus, may involve primarily component I flow, with the redistribution between components I and II during hemorrhage possibly involving other mechanisms.

Débit sanguin rénal au cours de la stimulation du nerf du sinus carotidien et de l'hémorragie chez le chien. Ce travail a été entrepris pour comparer, au cours de l'hémorragie, le rôle du système nerveux sympathique avec celui de l'hypotension dans le déterminisme de la redistribution du débit sanguin rénal (RBF). Dix chiens batards ont été préparés à l'étude de la distribution du débit sanguin rénal par l'injection de ^{85}Kr, en solution dans du soluté salé, dans l'artère rénale afin d'obtenir les courbes de radioactivité rénale. La distribution de RBF a été déterminée *a*) à l'état témoin, *b*) au cours d'une période d'hypotension de 15 minutes produite par la stimulation électrique (10 v—60 Hz) du nerf sino-carotidien gauche (nerf de Hering), *c*) 15 minutes après une hémorragie telle que la pression artérielle soit semblable à celle observée au cours de la stimulation et *d*) pendant une hémorragie associée à une période de stimulation de 15 minutes. L'hypotension déterminée par la stimulation n'affecte pas la composante I du RBF (approximativement 492 ml/min/100 g) mais entraîne une augmentation de la composante II du débit de 93 ± 8 à 155 ± 20 ml/min/100 g. L'hémorragie détermine une réduction de 60% de la composante I et n'affecte pas la composante II. Une réversibilité partielle des effets de l'hémorragie sur la redistribution est obtenue par la restimulation du nerf de Hering. Il apparaît que le contrôle de la distribution de RBF par le sinus carotidien intéresse essentiellement la composante I et que la redistribution entre composantes I et II au cours de l'hémorragie peut impliquer d'autres mécanismes.

Received for publication August 2, 1974; and in revised form February 21, 1975.

In our laboratory we have made extensive use of the technique of Thorburn et al [1] for the quantification of regional blood flow in the kidney. Carriere et al [2] used this technique, and Rector et al [3] used radioactive microsphere distribution to show that blood flow in the outer renal cortex approached the inner cortical-outer medullary rates during hemorrhagic hypotension. Aukland and Wolgast [4]—using red blood cell mean transit time for cortical flow rate, and hydrogen gas clearance for medullary flow rate—reported that both cortical and medullary flows were reduced proportionately, and that neither had any selective change in resistance in comparison to the other.

Pomeranz, Birtch and Barger [5] used the ^{85}Kr technique and activation of the renal nerve through carotid sinus baroreceptor activation. They found a decrease in volume perfusion for outer cortical flow as well as an increase in inner cortical-outer medullary flow. Sympathoadrenergic factors were also shown by Grandchamp, Ayer and Truniger [6] to be primarily responsible for cortical ischemia in hemorrhagic hypotension. In their [6] experiments, alpha-adrenergic blockade completely reversed redistribution of blood flow in hemorrhage. However, Carriere and Daigneault [7] found incomplete protection of the renal cortical flow with alpha blockage.

Other microsphere research [8] has revealed that renal blood flow redistribution may take place as a result of decreased renal perfusion pressure. Stein et al [9], using microspheres, found no change in hemorrhage-induced renal blood flow redistribution with alpha or beta blockade or atropine. Also, constant pressure perfusion of a kidney while the dog was hemorrhaged prevented redistribution of its blood flow. They [9] concluded that renal blood flow distribution was pressure-dependent. The pressure-dependent theory has been contradicted by Gransjo and Wolgast [10], who demonstrated similar changes for renal cortex and medulla during renal

circulatory hypotension produced by a suprarenal clamp on the aorta. They used red blood cell mean transit times and renal medullary hydrogen clearance to measure cortical and medullary blood flow rates, respectively.

To resolve the differences between renal nerve activity and direct perfusion pressure effects, we used carotid sinus nerve (nerve of Hering) electrical stimulation to produce a decrease in mean blood pressure coupled with a lack of reflex sympathetic tone [11]. The measurement of renal blood flow distribution during stimulation hypotension allowed us to determine the effects due primarily to decreased blood pressure. The effects of hypotension accompanied by increased vasoconstrictor tone were examined by hemorrhaging the animal to the same blood pressure as obtained by nerve-of-Hering stimulation.

Methods

Experimental procedure. Nine mongrel dogs (weighing 12 to 20 kg) were anesthetized with 5 mg/kg of morphine and 20 mg/kg of pentobarbital. The dogs had been starved for 12 hr but allowed water *ad lib.* The animals were then splenectomized and prepared for measurement of intrarenal blood flow as follows. Heparin (10,000 U) was administered as an anticoagulant. Through a flank incision, a polyvinyl cannula with a stainless steel tip was passed into the abdominal aorta and its tip extended just into the left renal artery [1]. To determine renal blood flow distribution, slug injections of approximately 500 μCi of ^{85}Kr (New England Nuclear) dissolved in saline (0.50 ml) were introduced through this cannula into the renal arterial circulation. The cannula was then flushed with saline solution as rapidly as possible. Control readings of renal blood flow distribution (^{85}Kr curve) and mean blood pressure were recorded. Following this the left carotid sinus nerve bundle was isolated by blunt dissection. The bulb-shaped carotid sinus was located at the bifurcation of the internal and external carotid arteries. The nerve fibers running rostrally from the surface of the carotid sinus were isolated and stimulating electrodes applied to them. This bundle (nerve of Hering) was stimulated for 15 min using approximately 10 v at 60 cycles/sec from a stimulator (Grass S44). During the period of hypotension caused by stimulation, a second ^{85}Kr curve was obtained. The next procedure was to remove the stimulus and bleed the dog into a reservoir to a mean blood pressure identical to that obtained during stimulation. Fifteen minutes after bleeding, a third ^{85}Kr curve and blood sample were obtained. In four dogs the stimulator was again turned on for 15 min during hemorrhage and a fourth ^{85}Kr curve was obtained.

Intrarenal blood flow determinations. Following an intraarterial slug injection of ^{85}Kr, an immediate increase, and later on an exponential decrease, in the radioactivity levels of the kidney were detected by a scintillation probe placed on a stand above the dog's side, aimed directly at the kidney. A ratemeter (Nuclear Chicago) converted the signal from the scintillation detector to an electronic signal which was recorded on a recorder (Electronics for Medicine DR8) as a logarithmic output.

The downslope of the radioactivity curve has been shown by Thorburn et al [1] to be a series of exponentials. The method most widely used to separate the downslope curve into its four basic components consists of a procedure in which components are successively fitted to the tail of the curve and subtracted from the remainder to obtain three or four components [1]. Components I, II, III and IV are generally accepted as representing flow through outer cortex, inner cortical-outer medullar, inner medulla and perirenal fat, respectively. The percentage of radioactivity going to each component was determined by finding the intercept of each with the ordinate.

The flow in any one component as determined by the slope of the line representing that component can be calculated as follows: Flow (ml/100 g/min) = $0.693 \times 100/t\frac{1}{2}$, where $t\frac{1}{2}$ is the length of time required for the radioactivity in the component to decrease by one-half its original value and 0.693 is $\log_e$ of 2. The derivation of the above relationship has been published previously [1]. All flow computations were done by a computer (IBM 1130).

Data for the first two components from ten dogs were compiled and standard t tests performed to determine if the mean value for flow in any component differed significantly from the mean flow in that component during any other phase of the experiment.

Results

During control conditions the mean blood pressure was 110 ± 2 mm Hg (mean ± SEM) (Fig. 1, Table 1). Upon application of the stimulus to the left nerve of Hering, there was a significant drop in mean blood pressure ($P < 0.001$) to 72 ± 3 mm Hg. The animals were then hemorrhaged to a mean pressure of 74 ± 3 mm Hg. During a second hemorrhage phase, the stimulus was reapplied to the left nerve of Hering and the mean blood pressure was 72 ± 4 mm Hg.

Figure 1 and Table 1 reveal that blood flow in component I (503 ± 56 ml/min/100 g) was unchanged

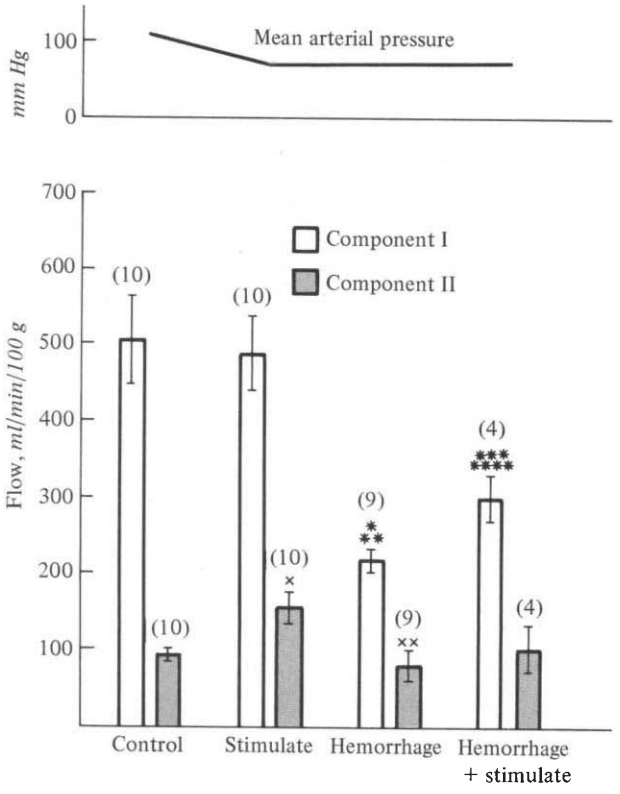

Fig. 1. *Composite of blood flow values for all dogs.* Each *bar* represents the mean (± SEM) for the flow components at each phase of the experiment. The number in parenthesis represents the number of dogs. Other symbols are as follows: * = hemorrhage < control ($P < 0.001$); ** = hemorrhage < stimulate ($P < 0.001$); *** = hemorrhage + stimulate > hemorrhage ($P < 0.05$); **** = hemorrhage + stimulate < control ($P < 0.01$); X = stimulate > control ($P < 0.05$); XX = hemorrhage < stimulate ($P < 0.01$).

during the hypotension produced by stimulating the nerve of Hering (492 ± 51 ml/min/100 g) but was reduced to less than one-half (221 ± 14 ml/min/100 g) of the control level ($P < 0.001$) during hemorrhage to the same mean blood pressure as was attained during stimulation. In all four cases where hemorrhage plus stimulation was tried, there was a marked increase ($P < 0.05$) above the value for hemorrhage alone in component I flow. However, the flow in this component did not rise back to control levels. Component II flow (93 ± 8 ml/min/100 g) was significantly ($P < 0.05$) increased by stimulation (155 ± 20 ml/min/100 g), but fell back to control levels (82 ± 16 ml/min/100 g) during hemorrhage. Restimulation during hemorrhage produced no change in component II.

Discussion

The object of the present study was to compare the relative roles of the sympathetic nervous system, as mediated by the carotid sinuses, and hypotension, accompanied by lack of sympathetic tone, in renal blood flow distribution. The methods used in this study for determination of intrarenal blood flow distribution were developed by Thorburn et al in 1963 [1]. This method, in contrast to most others, allows repetitive determinations during the course of an experiment lasting several hours and has been applied clinically in human patients.

There are significant criticisms that the several components of the inert gas washout curves may not actually represent flow in the renal areas described by Thorburn et al [1]. Slotkoff et al [12] have suggested that, when compared to blood flow distribution determined by radioactive microspheres, the first component seemed to be measuring cortical flow but the second component did not seem to represent inner cortical flow. Some researchers [9, 12] feel that the microsphere-measured renal blood flow distribution may delineate flow to individual cortical zones more specifically than the inert gas method. However, Jones and Herd [13] have done some excellent studies using serial autoradiographs, obtained by freezing and slicing kidneys at various intervals after ^{85}Kr injections. They found the initial distribution of ^{85}Kr to be approximately as expected from previous studies. The disappearance of radioactivity from the cortex was as rapid as would be expected, considering the rate of blood flow through the area. Their studies also revealed a set of peritubular capillaries in the outer layer of the medulla that arises directly from juxtamedullary efferent arterioles. The flow through these capillaries would likely be separate from and not too susceptible to the countercurrent exchange of the true vasa recta.

Previous studies [2] have indicated a change in anatomical distribution of these components during hemorrhagic hypotension. The primary finding of our studies was that there was no change in the flow rate in component I during stimulation hypotension when compared to the control curve. However, since we have no evidence that components I and II did not undergo changes in anatomic distribution, they are referred to only as components and no anatomic distinctions are made. It is assumed that the anatomical distribution of the components during the hemorrhagic hypotension phase was similar to that reported in previous studies [2].

During control conditions the renal blood flow distribution, in this study, agreed well with those reported in previous studies [1, 2, 4–7] (Fig. 1, Table 1). Components I and II had flow rates of 503 ± 56 and 93 ± 8 ml/100 g/min, respectively.

Within 15 min after the onset of stimulation

Table 1. Blood pressure and flow component data from dogs that were subjected to stimulation of the left carotid sinus nerve, hemorrhage and both together

	Control			Stimulation only		
Dog No.	Mean blood pressure *mm Hg*	Flow component I *ml/100 g/min*	Flow component II *ml/100 g/min*	Mean blood pressure *mm Hg*	Flow component I *ml/100 g/min*	Flow component II *ml/100 g/min*
6/12	110	324	93	70	416	220
6/15	105	680	72	60	423	96
6/23	120	852	126	75	747	280
6/30	110	345	86	65	351	146
7/6	100	461	106	75	759	137
7/12	115	410	70	70	499	133
7/13	110	542	115	65	377	104
7/21	110	495	109	90	412	145
7/24	110	450	57	80	441	133
Mean	110	503	93	72	492	155
SEM	2	56	8	3	51	20

hypotension, measurement of renal blood flow distribution revealed no change in component I flow in spite of the reduced perfusion pressure (Fig. 1). Therefore, renal vasodilation in this area must have exactly balanced any blood flow changes expected from the reduced pressure. When the animals were hemorrhaged to a degree of hypotension equivalent to that of carotid sinus stimulation in an effort to activate the sympathetic nervous system, a direct contrast to stimulation hypotension became apparent. The flow rate in component I decreased 60% (Fig. 1). This decrease agrees with those found in other hemorrhage experiments [2, 3, 6, 7, 9]. Restimulation of the carotid sinus nerve during hemorrhage resulted in a significant increase in flow (Fig. 1, Table 1) in component I. However, flow was lower than that found for stimulation alone, even though mean blood pressure was set at the same level as during stimulation alone. Therefore, it seems possible that effects of hemorrhage, other than pressure alone or carotid sinus-mediated reflexes alone, may cause some of the constriction in component I. This possibility has been suggested by others [7], and factors such as angiotensin II have been considered.

During the hypotension of Hering nerve stimulation, a significant increase in component II flow indicated that there may be even more vasodilation in this component than in component I which had the same flow rate after stimulation as before. An innervation of the blood vessels of the outer medulla by adrenergic nerve fibers has been demonstrated by Fourman [14]. Therefore, relaxation of sympathetic tone seems possible. However, this does not explain the maintenance of control levels of flow in component II during hemorrhage when sympathetic tone would be expected to be increased. The maintenance of control levels of flow for component II during hemorrhage agrees with previous findings [2, 3, 6, 7, 9]. When the stimulator was turned on again, during the hemorrhage phase, there was no significant difference in component II flow from that in any other phase of the experiment.

A change in renal blood flow distribution due to changes in renal perfusion pressure was indicated by McNay and Abe [8] and Stein et al [9]. It should be noted that the redistribution we found was due to an increase in component II, rather than a decrease in component I. The previous studies [8, 9] have indicated that the primary change was a decrease in outer cortical flow as determined by the use of the microsphere method. However, in our studies, this augmentation of component II could have been due either to the decrease in blood pressure or the decrease in sympathetic tone. No change in initial distribution of radioactivity was found in these studies.

During hemorrhagic hypotension we have demonstrated a reduced component I flow (Fig. 1, Table 1). This would appear to be in agreement with the findings of McNay and Abe [8] and Stein et al [9] as far as redistribution is concerned. However, our data seem to contradict their theory that perfusion pressure is more important than the sympathetic nervous system in controlling the flow in component I. That the sympathetic nervous system is primarily in control of nutrient flow in component I is evident when a comparison is made between the unaffected flow during stimulation hypotension and the ischemia of hemorrhagic hypotension.

Our data indicate that carotid sinus-mediated reflexes play a role in the control of flow in component I since carotid sinus stimulation hypotension alone did not affect its flow. Component II of the renal circulation may undergo a paradoxical increase

Hemorrhage only			Hemorrhage + stimulation		
Mean blood pressure *mm Hg*	Flow component I *ml/100 g/min*	Flow component II *ml/100 g/min*	Mean blood pressure *mm/Hg*	Flow component I *ml/100 g/min*	Flow component II *ml/100 g/min*
68	233	44			
75	243	123	75	319	71
78	178	68	78	370	149
70	183	61	60	242	33
77	189	189			
70	191	60			
65	218	33			
90	309	83			
77	244	77	75	269	162
74	221	82	72	300	103
3	14	16	4	28	31

in flow in the presence of the reduced perfusion pressure of hemorrhage or that of decreased sympathetic tone. This can be inferred from the fact that component II flow increased during stimulation in spite of the reduced blood pressure.

Acknowledgments

This investigation was supported by the Kentucky Heart Association, the Heart Association of Louisville and Jefferson County and GRS grant 583401G.

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References

1. THORBURN GD, KOPALD HH, HERD JA, HOLLENBERG M, O'MORCHOE CCC, BARGER AC: Intrarenal distribution of nutrient blood flow determined with krypton^{85} in the unanesthetized dog. *Circ Res* 13:290–307, 1963
2. CARRIERE S, THORBURN GD, O'MORCHOE CCC, BARGER AC: Intrarenal distribution of blood flow in dogs during hemorrhagic hypotension. *Circ Res* 19:167–179, 1966
3. RECTOR JB, STEIN JH, BAY WH, OSGOOD RW, FERRIS TF: Effect of hemorrhage and vasopressor agents on distribution of renal blood flow. *Am J Physiol* 222:1125–1131, 1972
4. AUKLAND K, WOLGAST M: Effect of hemorrhage and retransfusion on intrarenal distribution of blood flow in dogs. *J Clin Invest* 47:488–501, 1968
5. POMERANZ BH, BIRTCH AG, BARGER AC: Neural control of intrarenal blood flow. *Am J Physiol* 215:1067–1081, 1968
6. GRANDCHAMP A, AYER G, TRUNIGER B: Pathogenesis of redistribution of intrarenal blood flow in hemorrhagic hypotension. *Eur J Clin Invest* 1:271–276, 1971
7. CARRIERE S, DAIGNEAULT B: Effect of retransfusion after hemorrhagic hypotension on intrarenal distribution of blood flow in dogs. *J Clin Invest* 49:2205–2217, 1970
8. MCNAY JL, ABE Y: Pressure-dependent heterogeneity of renal cortical blood flow in dogs. *Circ Res* 27:571–587, 1970
9. STEIN JH, BOONJARERN S, MAUK RC, FERRIS TF: Mechanism of the redistribution of renal cortical blood flow during hemorrhagic hypotension in the dog. *J Clin Invest* 52:39–47, 1973
10. GRANSJO G, WOLGAST W: The pressure-flow relationship in renal cortical and medullary circulation. *Acta Physiol Scand* 85:228–236, 1972
11. VATNER SF, FRANKLIN D, VANCITTERS RL, BRAUNWALD E: Effects of cartoid sinus nerve stimulation on blood flow distribution in conscious dogs at rest and during exercise. *Circ Res* 27:495–503, 1970
12. SLOTKOFF LM, LOGAN A, JOSE P, D'AVILLA J, EISNER GM: Microsphere measurement of intrarenal circulation of the dog. *Circ Res* 28:158–166, 1971
13. JONES LG, HERD JA: Autoradiographic visualization of ^{85}Kr in the normal dog kidney. *Am J Physiol* 226:886–892, 1974
14. FOURMAN J: The adrenergic innervation of the efferent arterioles and the vasa recta in the mammalian kidney. *Experientia* 26:293–294, 1970